UK NEQAS for Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Paroxysmal Nocturnal Haemoglobinuria

Participant: 42981 **Trial No: 141504**

Date Issued: 02-March-2015 **Closing Date: 20-March-2015**

Your Results

Sample: 075

Trial Comments

This trial was issued to 158 participants worldwide.

Sample Comment
The sample was manufactured by UK NEQAS using a stabilised whole blood unit

Target Cells	Your Result	Median Result	Lower Quartile	Upper Quartile	Number of Returns
RBC PNH Clone - CD59		0.00	0.00	0.00	127
RBC Type II Cells - CD59		0.00	0.00	0.00	115
RBC Type III Cells - CD59		0.00	0.00	0.00	114
Granulocytes PNH Clone - CD16		0.00	0.00	0.00	34
Granulocytes PNH Clone - CD24		0.00	0.00	0.00	105
Granulocytes PNH Clone - CD55		0.00	0.00	0.00	19
Granulocytes PNH Clone - CD59		0.00	0.00	0.00	20
Granulocytes PNH Clone - CD66b		0.00	0.00	0.00	29
Granulocytes PNH Clone - FLAER		0.00	0.00	0.00	124

Performance Monitoring

Sample 075

	Consensus	Your Result	Classification	Running Classification
RBC	Clone Absent		Outwith Consensus	Red 3
WBC	Clone Absent		Outwith Consensus	Red 3

1 = All Samples Within Consensus across last 3 samples
2 = 1 Sample Outwith Consensus across last 3 samples
3 = 2 or more Samples Outwith Consensus across last 3 samples
= Not enough samples to determine Running Consensus

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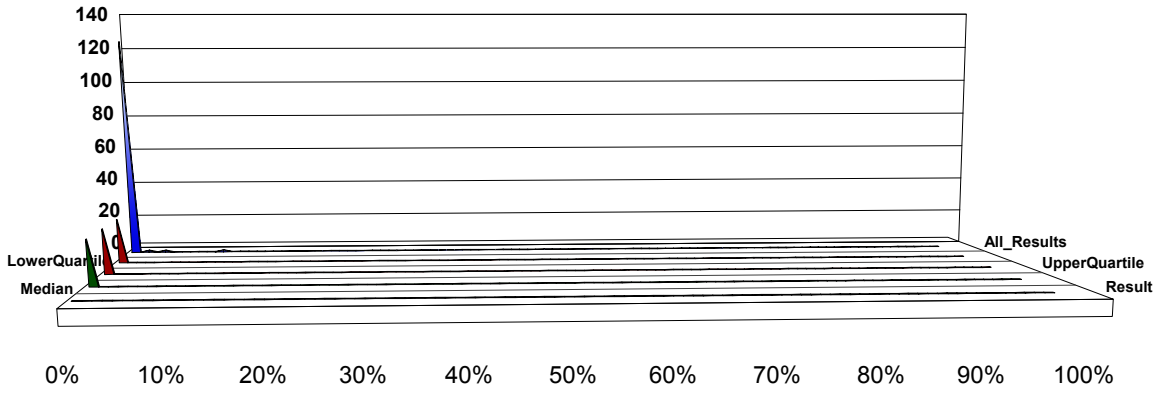
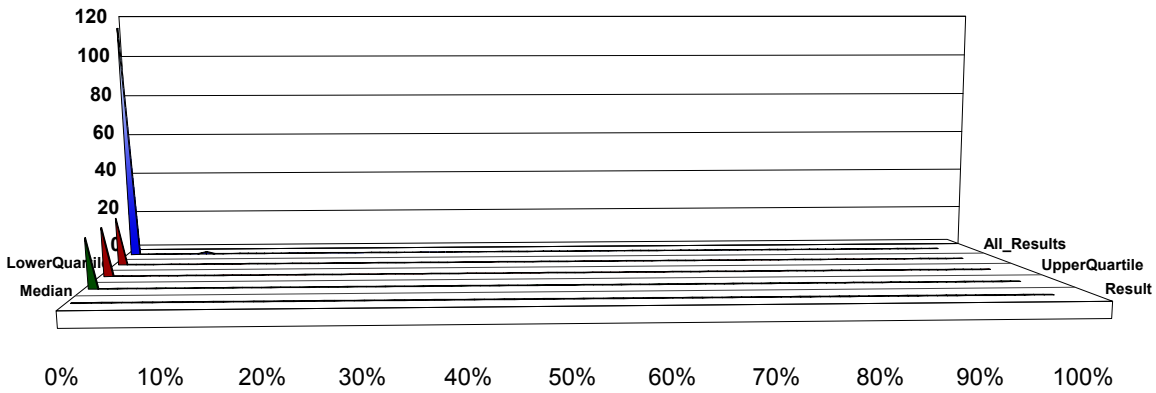
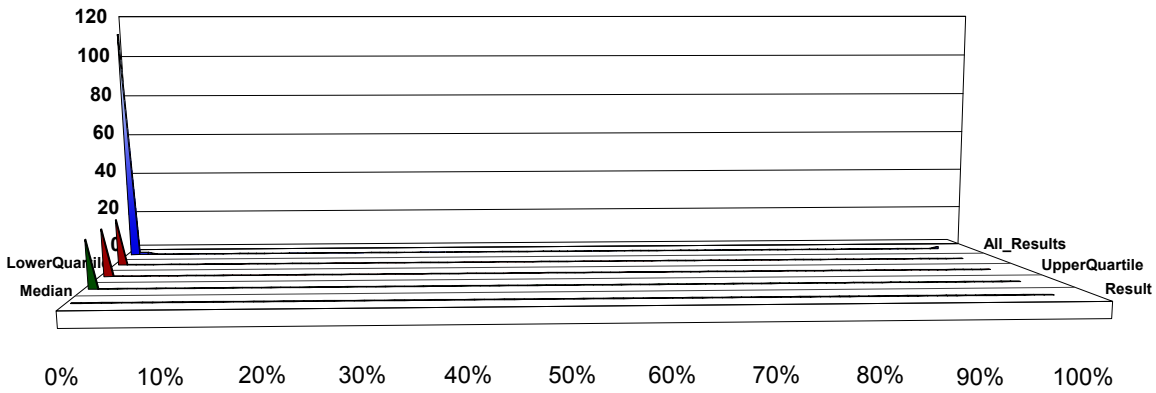
RBC PNH Clone - CD59



RBC Type II Cells - CD59



RBC Type III Cells - CD59



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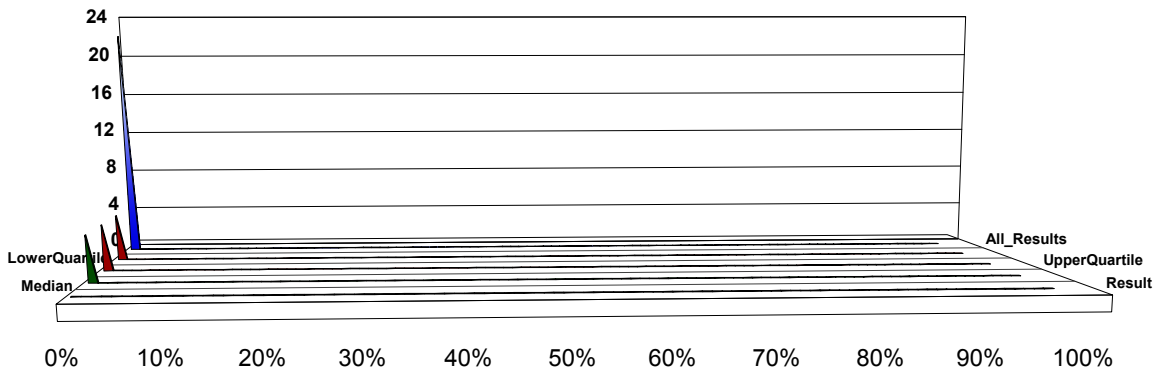
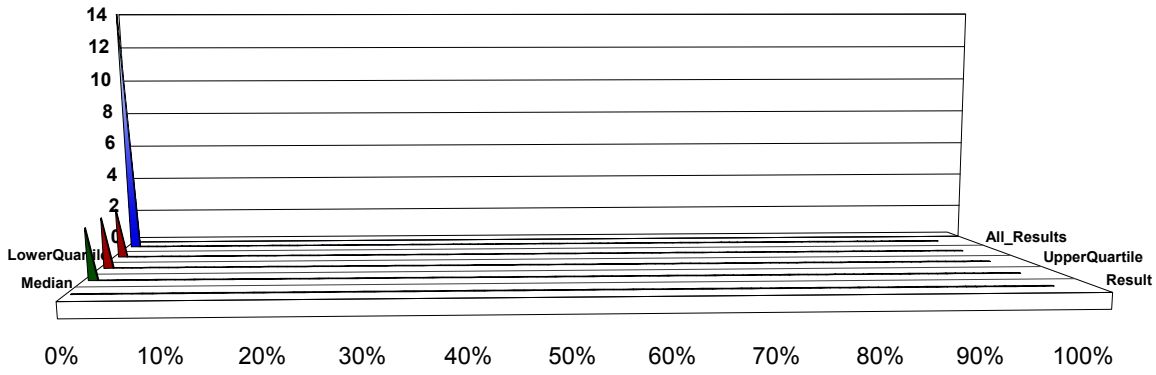
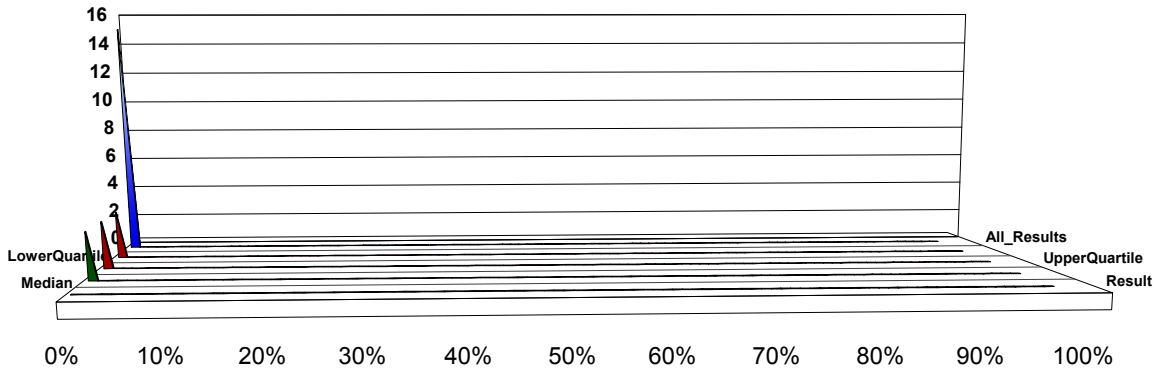
RBC PNH Clone - CD55



RBC Type II cells - CD55



RBC Type III cells - CD55



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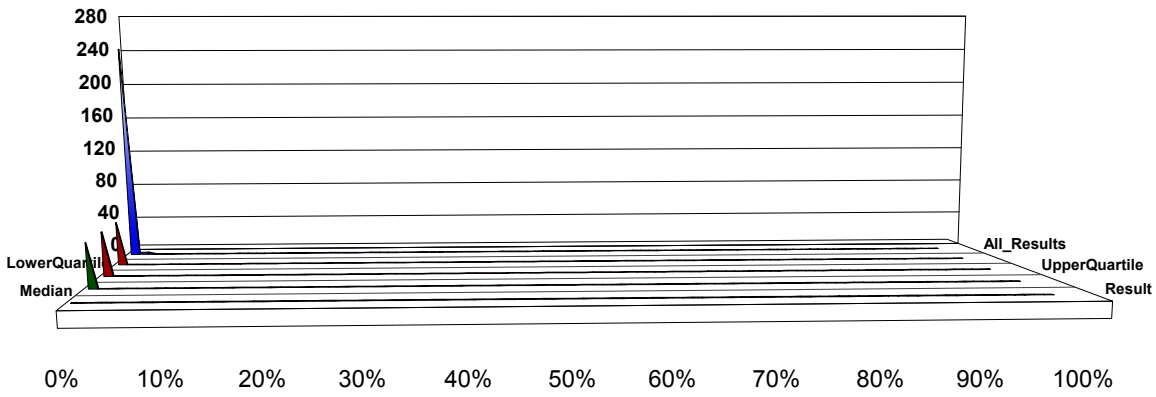
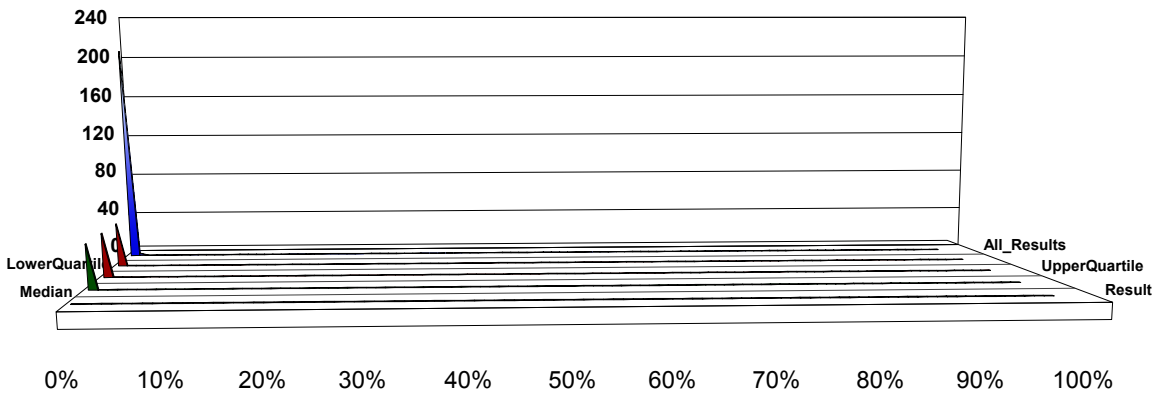
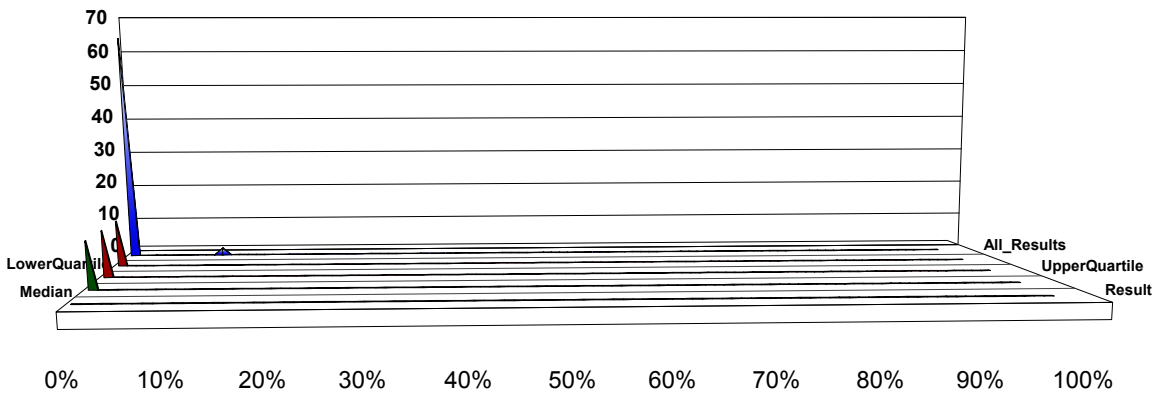
Granulocytes PNH Clone - FLAER



Granulocytes PNH Clone - CD24



Granulocytes PNH Clone - CD16



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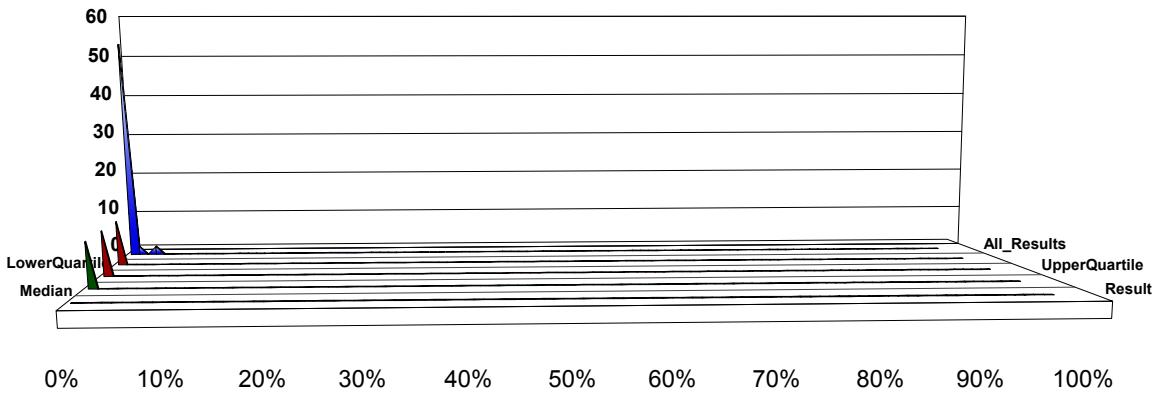
Granulocytes PNH Clone - CD66b



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Method Breakdown

RBC PNH Clone

Flow Cytometer	Median	Lower Quartile	Upper Quartile	Returns
CD55				
All	0.00	0.00	0.03	22
BD	0.00	0.00	0.04	17
CD59				
All	0.00	0.00	0.00	127
BD	0.00	0.00	0.00	87
Coulter	0.00	0.00	0.00	39

Antibody Manufacturer	Median	Lower Quartile	Upper Quartile	Returns
CD55				
All	0.00	0.00	0.03	22
BD Biosciences	0.00	0.00	0.32	11
CD59				
All	0.00	0.00	0.00	127
BD Biosciences	0.00	0.00	0.07	19
invitrogen	0.00	0.00	0.00	67

Fluorochrome	Median	Lower Quartile	Upper Quartile	Returns
CD55				
All	0.00	0.00	0.03	22
PE	0.00	0.00	0.30	15
CD59				
All	0.00	0.00	0.00	127
PE	0.00	0.00	0.00	99
FITC	0.00	0.00	0.01	27

CPA

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Method Breakdown
Granulocytes PNH Clone

Flow Cytometer	Median	Lower Quartile	Upper Quartile	Returns
CD16				
All	0.00	0.00	0.00	34
BD	0.00	0.00	0.00	25
CD24				
All	0.00	0.00	0.00	105
BD	0.00	0.00	0.00	72
Coulter	0.00	0.00	0.00	32
CD55				
All	0.00	0.00	0.00	19
BD	0.00	0.00	0.00	10
CD59				
All	0.00	0.00	0.00	20
BD	0.00	0.00	0.05	11
CD66b				
All	0.00	0.00	0.00	29
BD	0.00	0.00	0.00	21
FLAER				
All	0.00	0.00	0.00	124
BD	0.00	0.00	0.00	86
Coulter	0.00	0.00	0.00	37

Antibody Manufacturer	Median	Lower Quartile	Upper Quartile	Returns
CD16				
All	0.00	0.00	0.00	34
BD Biosciences	0.00	0.00	0.00	13
Beckman Coulter	0.00	0.00	0.04	11
CD24				
All	0.00	0.00	0.00	105
BD Biosciences	0.00	0.00	0.00	29
Beckman Coulter	0.00	0.00	0.00	48
Pharmingen	0.00	0.00	0.00	20
CD55				
All	0.00	0.00	0.00	19
CD59				
All	0.00	0.00	0.00	20
CD66b				
All	0.00	0.00	0.00	29
BD Biosciences	0.00	0.00	0.00	11
FLAER				
All	0.00	0.00	0.00	123
Other	0.00	0.00	0.00	61
Protox	0.00	0.00	0.00	52

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Fluorochrome	Median	Lower Quartile	Upper Quartile	Returns
CD16				
All	0.00	0.00	0.00	34
CD24				
All	0.00	0.00	0.00	105
PE	0.00	0.00	0.00	76
CD55				
All	0.00	0.00	0.00	19
PE	0.00	0.00	0.00	12
CD59				
All	0.00	0.00	0.00	20
PE	0.00	0.00	0.00	14
CD66b				
All	0.00	0.00	0.00	29
FITC	0.00	0.00	0.00	23
FLAER				
All	0.00	0.00	0.00	124
FITC	0.00	0.00	0.00	39
AlexaFluor 488	0.00	0.00	0.00	85

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Paroxysmal Nocturnal Haemoglobinuria

Participant: 42981 **Trial No: 141504**

Date Issued: 02-March-2015 **Closing Date: 20-March-2015**

Your Results

Sample: 076

Trial Comments

This trial was issued to 158 participants worldwide.

Sample Comment
The sample was manufactured by UK NEQAS using a stabilised whole blood unit

Target Cells	Your Result	Median Result	Lower Quartile	Upper Quartile	Number of Returns
RBC PNH Clone - CD59		0.00	0.00	0.00	126
RBC Type II Cells - CD59		0.00	0.00	0.00	114
RBC Type III Cells - CD59		0.00	0.00	0.00	113
Granulocytes PNH Clone - CD16		0.00	0.00	0.00	34
Granulocytes PNH Clone - CD24		0.00	0.00	0.00	104
Granulocytes PNH Clone - CD55		0.00	0.00	0.03	19
Granulocytes PNH Clone - CD59		0.00	0.00	0.03	20
Granulocytes PNH Clone - CD66b		0.00	0.00	0.00	28
Granulocytes PNH Clone - FLAER		0.00	0.00	0.00	123

Performance Monitoring

Sample 076

	Consensus	Your Result	Classification	Running Classification
RBC	Clone Absent		Outwith Consensus	Red 3
WBC	Clone Absent		Outwith Consensus	Red 3

1 = All Samples Within Consensus across last 3 samples
2 = 1 Sample Outwith Consensus across last 3 samples
3 = 2 or more Samples Outwith Consensus across last 3 samples
= Not enough samples to determine Running Consensus

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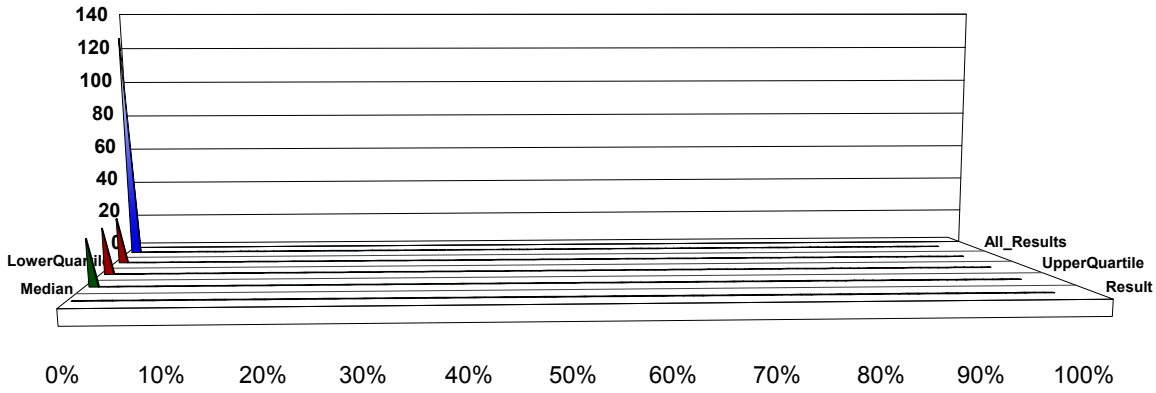
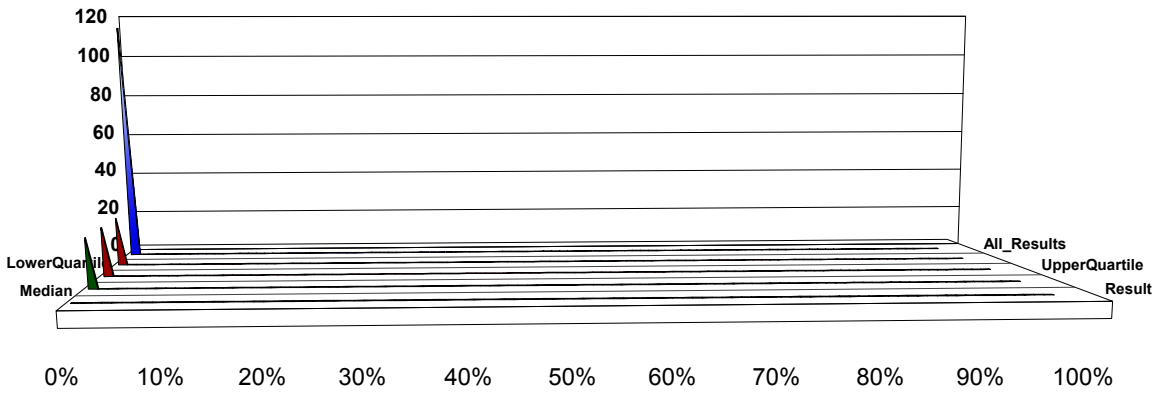
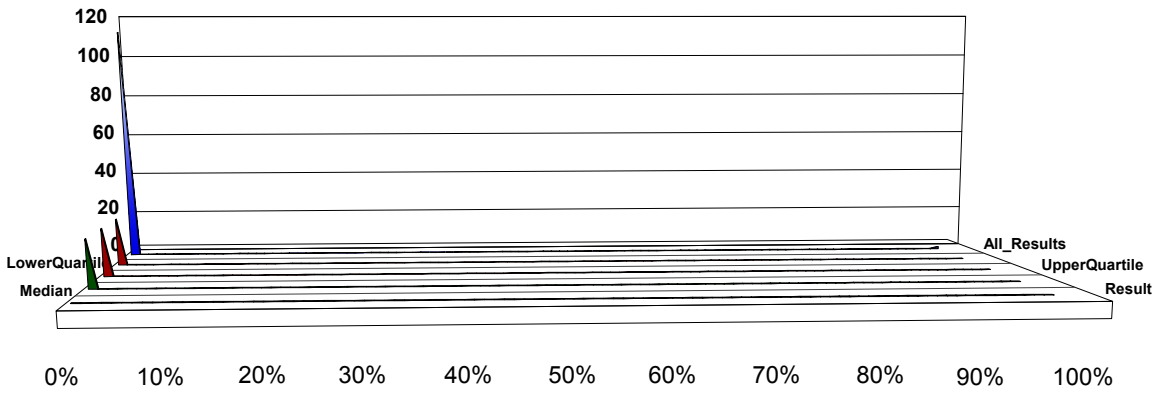
RBC PNH Clone - CD59



RBC Type II Cells - CD59



RBC Type III Cells - CD59



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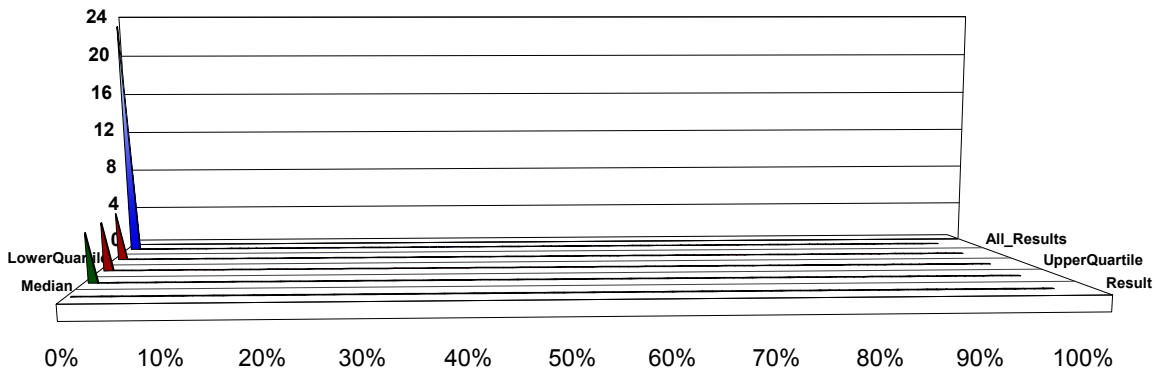
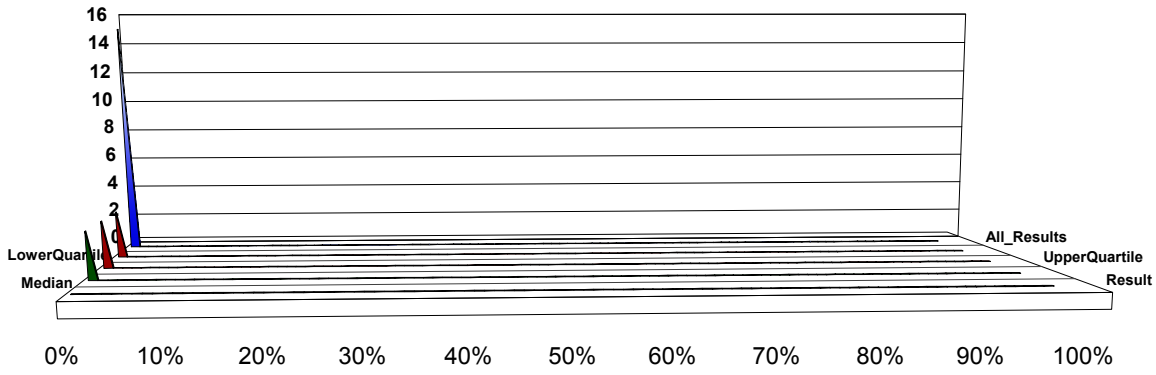
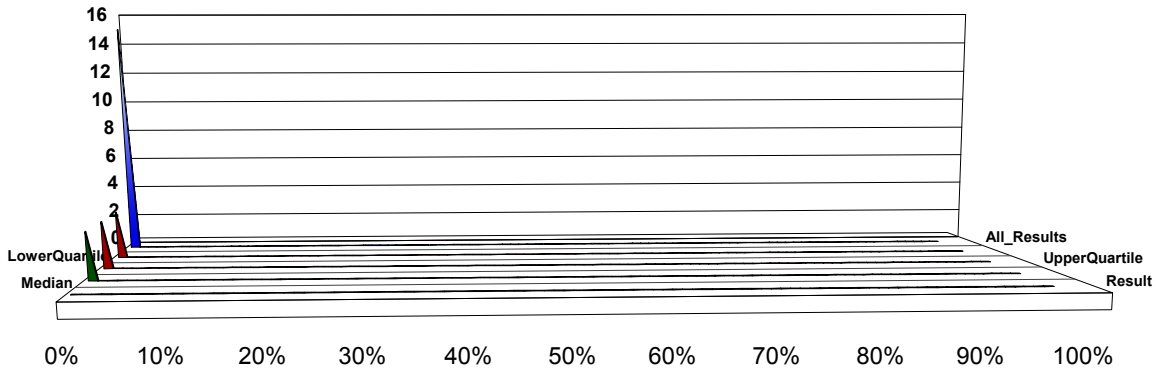
RBC PNH Clone - CD55



RBC Type II cells - CD55



RBC Type III cells - CD55



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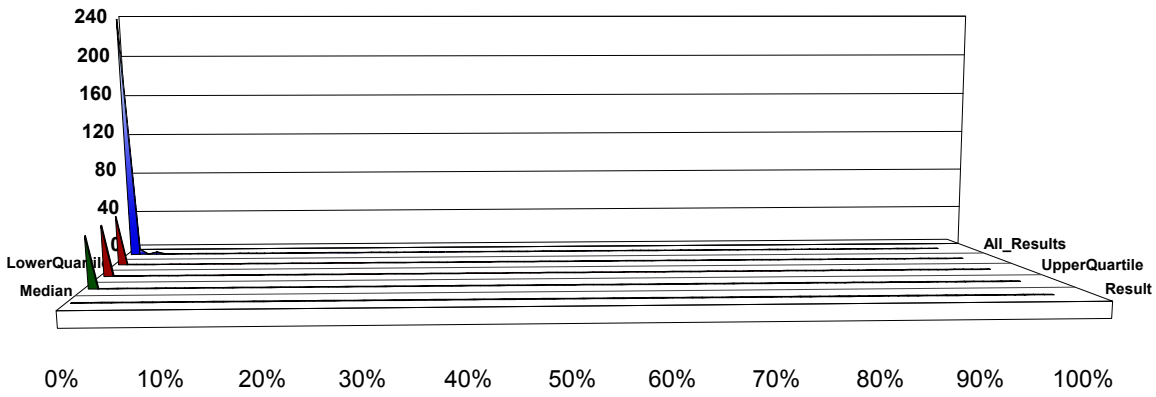
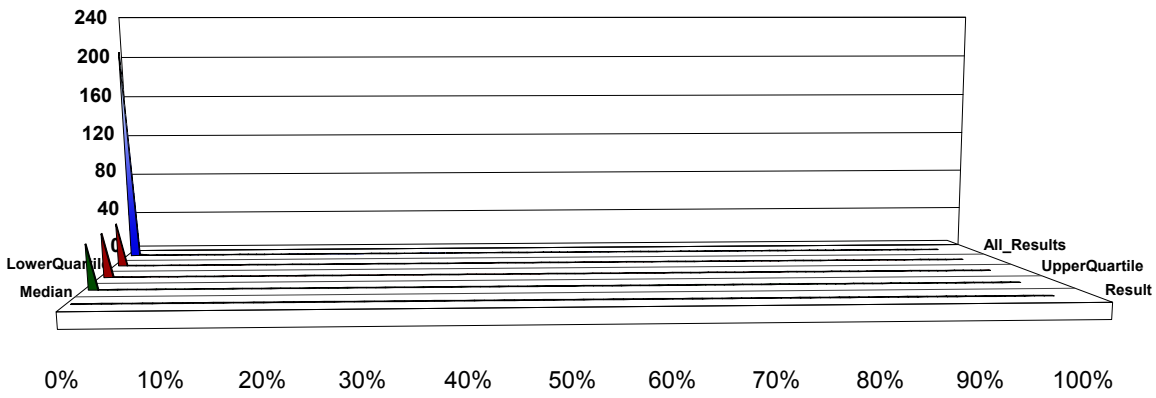
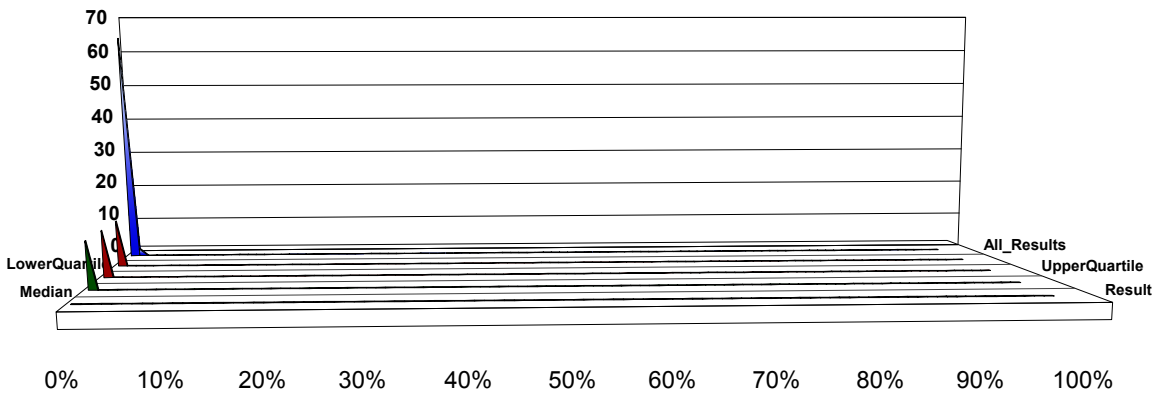
Granulocytes PNH Clone - FLAER



Granulocytes PNH Clone - CD24



Granulocytes PNH Clone - CD16



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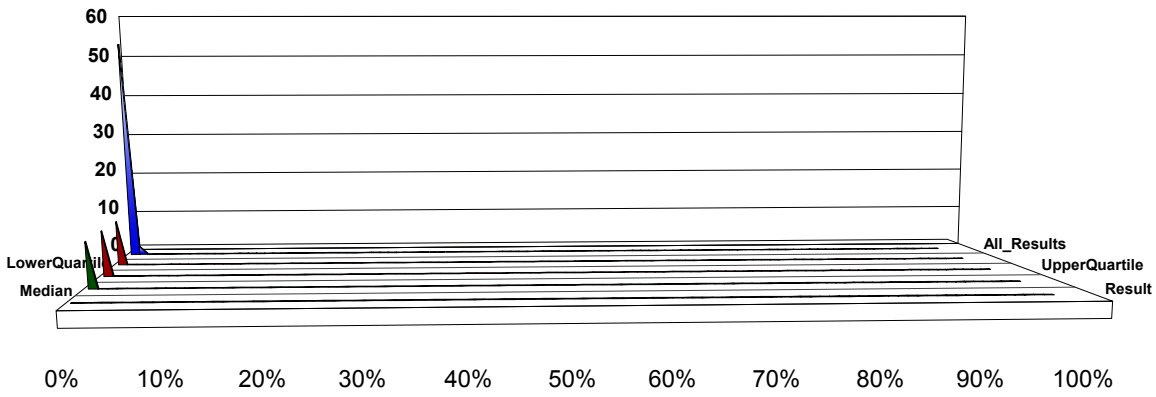
Granulocytes PNH Clone - CD66b



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Method Breakdown

RBC PNH Clone

Flow Cytometer	Median	Lower Quartile	Upper Quartile	Returns
CD55				
All	0.00	0.00	0.15	23
BD	0.00	0.00	0.18	18
CD59				
All	0.00	0.00	0.00	126
BD	0.00	0.00	0.00	86
Coulter	0.00	0.00	0.00	39

Antibody Manufacturer	Median	Lower Quartile	Upper Quartile	Returns
CD55				
All	0.00	0.00	0.15	23
BD Biosciences	0.00	0.00	0.24	11
CD59				
All	0.00	0.00	0.00	126
BD Biosciences	0.00	0.00	0.05	19
invitrogen	0.00	0.00	0.00	67

Fluorochrome	Median	Lower Quartile	Upper Quartile	Returns
CD55				
All	0.00	0.00	0.15	23
PE	0.00	0.00	0.30	16
CD59				
All	0.00	0.00	0.00	126
PE	0.00	0.00	0.00	98
FITC	0.00	0.00	0.00	27

CPA

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Method Breakdown
Granulocytes PNH Clone

Flow Cytometer	Median	Lower Quartile	Upper Quartile	Returns
CD16				
All	0.00	0.00	0.00	34
BD	0.00	0.00	0.00	25
CD24				
All	0.00	0.00	0.00	104
BD	0.00	0.00	0.00	72
Coulter	0.00	0.00	0.02	31
CD55				
All	0.00	0.00	0.03	19
BD	0.00	0.00	0.09	10
CD59				
All	0.00	0.00	0.03	20
BD	0.00	0.00	0.19	11
CD66b				
All	0.00	0.00	0.00	28
BD	0.00	0.00	0.00	20
FLAER				
All	0.00	0.00	0.00	123
BD	0.00	0.00	0.00	85
Coulter	0.00	0.00	0.00	37

Antibody Manufacturer	Median	Lower Quartile	Upper Quartile	Returns
CD16				
All	0.00	0.00	0.00	34
BD Biosciences	0.00	0.00	0.00	13
Beckman Coulter	0.00	0.00	0.18	11
CD24				
All	0.00	0.00	0.00	104
BD Biosciences	0.00	0.00	0.00	28
Beckman Coulter	0.00	0.00	0.00	48
Pharmingen	0.00	0.00	0.00	20
CD55				
All	0.00	0.00	0.03	19
CD59				
All	0.00	0.00	0.03	20
CD66b				
All	0.00	0.00	0.00	28
BD Biosciences	0.00	0.00	0.00	10
FLAER				
All	0.00	0.00	0.00	122
Other	0.00	0.00	0.00	60
Protox	0.00	0.00	0.00	52

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Fluorochrome	Median	Lower Quartile	Upper Quartile	Returns
CD16				
All	0.00	0.00	0.00	34
CD24				
All	0.00	0.00	0.00	104
PE	0.00	0.00	0.00	75
CD55				
All	0.00	0.00	0.03	19
PE	0.00	0.00	0.06	12
CD59				
All	0.00	0.00	0.03	20
PE	0.00	0.00	0.00	14
CD66b				
All	0.00	0.00	0.00	28
FITC	0.00	0.00	0.00	22
FLAER				
All	0.00	0.00	0.00	123
FITC	0.00	0.00	0.00	39
AlexaFluor 488	0.00	0.00	0.00	84

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PNH 141504

Sample 075

This sample was normal whole blood containing no PNH clone; the median values of the results returned reflected those obtained at pre-issue testing.

The consensus result for this sample was **Clone Absent** for both Red Cell and Granulocyte clones. 19 participants recorded the presence of a small red cell clone, 16 of these then reported the results as clone absent (0.01%-0.53%), the other 3 centres reported their results as clone present (clone size 0.1%, 2.5% and 11.5%).

22 participants recorded a small granulocyte clone on at least one of their markers, 15 of these were reported as being clone absent (clone size 0.001% to 11.5%), whilst 7 centres reported the results as clone present (0.035%-3.3%). This raises questions as to the levels of sensitivity being used for PNH clone determination

8 centres did not return results for this sample.

Sample 076

This sample was a duplicate of sample 075 and as such contained no PNH clone.

The consensus result for this sample was **Clone Absent** for both Red Cell and Granulocyte clones. 19 participants recorded the presence of a small red cell clone with 16 of these then reported as clone absent (clone size 0.003%-0.93%), the remaining 3 centres reported the results as being clone present (0.01%, 0.1% and 0.7%)

The 3 centres which identified a red cell clone in this sample were different to the 3 centres identifying a red cell clone in sample 075. Therefore, a total of six centres reported one sample as being clone present and the other as clone absent when testing duplicate material. Additionally, 2 of the centres which reported clone present on red cells reported clone absent on granulocytes.

25 participants recorded a small granulocyte clone on at least one of their markers, 18 of these were reported as being clone absent (clone size 0.001% to 1.5%), whilst 7 centres reported the results as clone present (0.05%-3.4%). 3 of the 7 centres had also incorrectly reported clone present on the other sample. The remaining 4 centres that identified a granulocyte clone in this sample were different to those identifying a clone in sample 075, therefore a total of 8 centres reported one sample as being granulocyte clone present and the other as granulocyte clone absent when testing duplicate material.

Two centres recorded granulocyte PNH clones at a level of 0.001% and 0.003%, for these results to have any statistical confidence would require the collection of at least 5 million events.

8 centres did not return results for this sample.

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Information with respect to compliance with standards BS EN ISO/IEC 17043:2010

4.8.2 a) The proficiency testing provider for this programme is:
UK NEQAS for Leucocyte Immunophenotyping
Pegasus House, 4th Floor Suite
463A Glossop Road
Sheffield, S10 2QD
United Kingdom
Tel: +44 (0) 114 267 3600, Fax: +44 (0) 114 267 3601
e-mail: nicola.rose@ukneqasli.co.uk

4.8.2 b) The coordinators of UK NEQAS LI programmes are Prof David Barnett and Mr Liam Whitby.

4.8.2 c) Person(s) authorizing this report:
Prof David Barnett, Director or Mr Liam Whitby, Operations Manager of UK NEQAS LI

4.8.2 d) No activities in relation to this EQA exercise were subcontracted.

4.8.2 g) The UK NEQAS LI Confidentiality Policy can be found in the Quality Manual which is available by contacting the UK NEQAS LI office. Participant details, their results and their performance data remain confidential unless revealed to the relevant NQAAP when a UK participant is identified as having performance issues.

4.8.2 i) All EQA samples are prepared in accordance with strict Standard Operational Procedures by trained personnel proven to ensure homogeneity and stability. Where appropriate/possible EQA samples are tested prior to issue. Where the sample(s) issued is stabilised blood or platelets, pre and post stability testing will have proved sample suitability prior to issue.

4.8.2 l), n), o), r) & s) Please refer to the UK NEQAS LI website at www.ukneqasli.co.uk for detailed information on each programme including the scoring systems applied to assess performance. Where a scoring system refers to the 'consensus result' this means the result reported by the majority of participants for that trial issue. Advice on the interpretation of statistical analyses and the criteria on which performance is measured is also given. Please note that where different methods/procedures are used by different groups of participants these may be displayed within your report, but the same scoring system is applied to all participants irrespective of method/procedure used.

4.8.2 m) We do not assign values against reference materials or calibrants.

4.8.2 q) Details of the programme designs as authorized by The Steering Committee and Specialist Advisory Group can be found on our website at www.ukneqasli.co.uk. The proposed trial issue schedule for each programme is also available.

4.8.2 t) If you would like to discuss the outcomes of this trial issue, please contact UK NEQAS LI using the contact details provided.

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